ENVIRONMENTAL RESEARCH

RESEARCH AND TECHNOLOGY BRANCH

Environment
Ontario

ISBN 0-7729-5944-7

92-01651/

ALGAL COMPETITION IN STEADY STATE CONTINUOUS CULTURE: RESPONSE OF ANABAENA AND SCENEDESMUS TO VARIATIONS IN CO_2 AND IRON

R. A. C. PROJECT NO. 337 RR

Report prepared for Environment Ontario by:

Dr. Lewis Molot
Molot Environmental Services Inc.
and
Dr. C.G. Trick
Department of Plant Sciences
University Of Western Ontario

AUGUST 1989
Reprinted August 1991

Copyright: Queen's Printer for Ontario, 1989

DISCLAIMER

This report has been reviewed by the Research Advisory Committee of Environment Ontario and approved for publication. Approval does not necessarily signify that the contents reflect the views nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

SUMMARY

A novel continuous culture apparatus was designed to maintain indefinite competition between two algal species via a constant inflow of both species from monospecific cultures into a competition vessel. The apparatus was used to test the hypothesis that phytoplankton communities in eutrophic waters are dominated by nuisance blue-green algae (Cyanophyta) because of the effect of decreasing surficial sediment redox as the summer progresses on water chemistry in the water column (increasing Fe, decreasing CO_2). We simulated oligotrophic (high CO_2, low Fe) and eutrophic conditions (low CO_2, high Fe) in laboratory microcosms in attempts to manipulate the outcome of competition experiments between a cyanophyte, Anabaena variabilis, and a chlorophyte, Scenedesmus sp.

The continuous flow competition apparatus demonstrated its ability to maintain coexistence of two species. Hence, this apparatus can be used to study the nature of competitive interactions for indefinite, stable periods rather than short-lived transient periods. A preliminary theoretical framework for growth kinetics in dual species continuous culture is provided.

Screening experiments indicated that Scenedesmus growth rates were higher than Anabaena growth rates only at high CO_2 and low Fe which is consistent with the sediment redox hypothesis.

In longer term experiments, Anabaena growth exceeded that of Scenedesmus at high CO_2 and high Fe, and at low CO_2 and low Fe. Additional long term experiments are currently underway to test the remaining treatments - high CO_2 with low Fe, and low CO_2 with high Fe. These experiments are necessary to verify the results of the screening experiments at high CO_2 in which Scenedesmus appears to have won.

Allelopathic interactions were discounted after paper disks saturated with filtrate from Anabaena culture failed to inhibit Scenedesmus growth on solid substrate culture (agar plates with BG-11 media).

INTRODUCTION

In an interim report for this project submitted in March of 1988, the preliminary results of a novel steady state continuous culture apparatus were presented. We attempted to test the hypothesis that the redox potential of surficial lake sediments indirectly controls the relative abundance of nuisance blue-green algae (Cyanophyta). It was hypothesized that phytoplankton communities are dominated by nuisance blue-green algae in eutrophic waters because of the effect of decreasing sediment redox on concentrations of Fe, CO_2 or other substances as the summer progresses. Hence, we simulated oligotrophic (high CO_2, low Fe) and eutrophic (low CO_2, high Fe) conditions in laboratory microcosms in attempts to manipulate the outcome of competition experiments between a cyanophyte, Anabaena variabilis, and a chlorophyte, Scenedesmus sp.

The novel feature of our continuous culture apparatus is its ability to maintain indefinite competition via a constant inflow of both species from monospecific cultures into a competition vessel. Conventional continuous flow competition studies do not have constant inflows from upstream cultures; hence, one species washes out when growth of both is limited by the same resource, something which is an unlikely event in natural aquatic systems.

Preliminary work centred first on unsuccessful attempts to isolate the cyanophyte Microcystis axenically. After choosing to work with Anabaena, the next step was to "debug" the new continuous culture apparatus. We reported last year that co-existence of Anabaena and Scenedesmus was successfully achieved and maintained but that more work was needed to test our hypothesis of redox-mediated control of species composition. This report presents the results of further design modifications and additional experiments at several combinations of high and low CO_2 and Fe at one dilution rate.

METHODS

The continuous culture apparatus was a dual phase (air and water), three stage system in which effluent from two monospecific cultures converged downstream in a third growth vessel (Figure 1). The effluent converged first in a mixing chamber to vent off excess gas and to occasionally reduce some of the effluent. The constant input of cells from upstream monospecific cultures to a competition chamber creates steady state competition, i.e. ongoing competition which is time-independent. As a general guideline, steady state begins after a

minimum of 3 residence times where the residence time is defined as culture volume/flushing rate (i.e, steady state is reached after 9 days at a residence time of 3 days or a dilution rate of 0.33/day).

Media was pumped with a multihead peristaltic LKB Multiperpex pump. The growth vessels were water-jacketed to control temperatures which varied between 22-24 °C. Constant incident light radiation was provided by fluorescent lamps at 80-90 $uE/m^2/sec$.

Stirring rate and air flow were constant in all vessels. The air was bubbled through sterile, distilled water to saturate with moisture. CO_2 was added to the air stream at 0, 0.1, 1 or 2% of the air flow rate.

Algal cultures were grown in BG-11 media (Rippka et al. 1979) without Na_2CO_3 and modified with Tris buffer. The molar N/P ratio was 30. Two Fe concentrations were used; the high total Fe concentration was 37.0 uM (2.10 mg Fe/L; pFe = 17) and the low Fe concentration was pFe = 19. pFe is defined as the equilibrium concentration of Fe^{+3} in fresh media and was calculated using the chemical equilibrium model MINEQL (Morel et al. 1979). The pH after autoclaving was 8.1.

Two axenic algal species were grown - the blue-green alga Anabaena variabilis and the chlorophyte Scenedesmus sp. (University of Toronto culture collection).

To initiate culturing, monospecific growth vessels were filled with 450 ml of media, inoculated and left in batch mode. When cultures were visibly ready, the pump was turned on and the volumes were decreased to 300 ml to supply an adequate start-up volume downstream. The volume of the competition culture was 900 ml. All dilution rates were 0.33 per day.

In the first series of experiments (series "A"), the influent rate from each monospecific culture was 100 ml/day and fresh media was supplied at a rate of 100 ml/day. Cultures were sparged with air or air enriched to 0.1 and 1% CO_2. In series "B", 70 ml of effluent from each monospecific culture was discarded. The influent rate from each monospecific culture into the competition vessel was 30 ml/day and the fresh media supply rate was 240 ml/day. Cultures were sparged with air or air enriched to 2% CO_2. Effluent diversion was implemented in series "B" to minimize the impact of fluctuations in upstream cell concentrations on cell concentrations in the competition vessel.

Aliquots were sampled through a sterile sampling port with a syringe and preserved with Lugol. Individual cells or filaments were counted with a haemocytometer.

Allelopathic inhibition of Scenedesmus growth by Anabaena was examined by placing paper disks saturated with filtrate from Anabaena culture on solid substrate culture (agar plates with BG-11 media) inoculated with Scenedesmus.

GROWTH KINETIC THEORY

Let X_1 = cell conc'n in monospecific culture of species X

Y_2 = " species Y

X_3 = cell conc'n in dual species culture of species X

Y_3 = " species Y

U_{x1} = growth rate of species X in mono culture

U_{x3} = " X in dual species culture

U_{y2} = " Y in mono culture

U_{y3} = " Y in dual species culture

$F_{1,2}$ = influent rate from each upstream vessel to competition vessel (ml/day)

$V_{1,2}$ = volume of both upstream vessels (ml)

F_3 = flushing rate of dual species vessel

= $F_1+F_2+F_m$ where F_m is the input rate of fresh media

V_3 = volume of dual species, competition culture

D = dilution rate in competition vessel

= F_3/V_3

Then the rates of change in cell concentrations in the downstream vessel are,

$$dX_3/dt = F_1*X_1/V_3 + (U_{x3} - D)*X_3 \quad (1)$$

$$dY_3/dt = F_2*Y_2/V_3 + (U_{y3} - D)*Y_3 \quad (2)$$

and $$U_{x3} = (dX_3/dt)(1/X_3) + D - (F_1/V_3)*(X_1/X_3) \quad (3)$$

$$U_{y3} = (dY_3/dt)(1/Y_3) + D - (F_2/V_3)*(Y_2/Y_3) \quad (4)$$

Hence, the growth rates in dual species culture can be calculated if the rates, F_i, and the cell concentrations in monospecific and competition cultures are known.

Theoretically, if species Y cannot compete with species X, then $U_{x3} > U_{y3}$. In conventional competition experiments without cell replacement, $U_{y3} < D$ and U_{x3} is close to D, hence, species Y washes out of the competition vessel permitting species X to establish itself at a growth rate of D. In experiments with cell replacement, it is conceivable that both growth rates will be > 0 but that one growth rate will be significantly greater.

RESULTS AND DISCUSSION

Monospecific Culture:

In series "A", Scenedesmus abundance was stable at both high and low Fe when sparged with air or 0.1% CO_2 but population fluctuations occurred more at 1% CO_2 (Figures 2-3). In series "B", Scenedesmus abundance was stable in air at low Fe and in air enriched to 2% CO_2 at high Fe (Figures 4-7).

Similarly, growth of Anabaena was stable at both high and low Fe when sparged with air or 0.1% CO_2. Sparging with 1% CO_2 appeared to destabilize populations at low Fe but not at high Fe (Figures 2-3). Growth was stable in series "B" (Figures 4-7).

Anabaena produced heterocysts at low Fe. Apparently, low Fe results in a deficiency in nitrate reductase when the sole N source is nitrate. This, in turn, causes N deficiency even in the presence of excess nitrate (unpublished data from Alison Kerry, M.Sc thesis, University of Toronto) which apparently induces heterocyst formation.

Competition Culture:

The competition experiment results are best analyzed by comparing growth rates rather than abundance. Growth rates are corrected for changes in cell concentrations caused by upstream fluctuations and permit monitoring of performance in the competition vessel.

In series "A" experiments without diversion of monospecific effluent, CO_2 enrichment was increased from 0% to 0.1% at 100 hr and then to 1% at 354 hr (Figures 8 and 9). The time period at each CO_2 level was relatively brief but allowed responses to six treatments to be screened. Anabaena growth rates exceeded Scenedesmus growth rates at 0% and 0.1% CO_2 at both Fe concentrations with the difference most pronounced at high Fe (pFe 17). Scenedesmus growth rates exceeded Anabaena at the highest CO_2 level (1%) and the difference was most pronounced at low Fe (pFe 19). Hence, in series "A", Scenedesmus fared best at simulated oligotrophic conditions - high CO_2 and low Fe - whereas Anabaena fared best at simulated eutrophic conditions - low CO_2 and high Fe.

The series "B" experiments were intended to follow up on the series "A" experiments. Four treatments were chosen to run for longer periods but with diversion of some monospecific effluent from the competition vessel. They are incomplete at present (our technician has been on a temporary leave of absence for several months) but two treatments have been completed in duplicate (Figures 9 and 10). At 0% CO_2 and low Fe, Anabaena growth rate averaged 0.28 and 0.23 per day and Scenedesmus growth rate averaged 0.25 and 0.15 per day after 12 days. At 2% CO_2 and high Fe, Anabaena growth rate averaged 0.30 and 0.28 per day and Scenedesmus growth rate averaged 0.22 and 0.13 per day after 15 days. In both cases, the growth rate of Anabaena was similar to the dilution rate and exceeded that of Scenedesmus. These results suggest that Scenedesmus would wash out if cell replacement were discontinued.

Anabaena's ability to dominate Scenedesmus raised suspicions that dominance was due to allelopathy rather than resource utilization. This was discounted after paper disks saturated with filtrate from Anabaena culture failed to inhibit Scenedesmus growth on solid substrate culture (agar plates with BG-11 media).

CONCLUSIONS

The continuous flow competition apparatus has demonstrated its ability to maintain coexistence of two species. Hence, this apparatus can be used to study the nature of competitive interactions for indefinite, stable periods rather than short-lived transient periods. The growth kinetics discussed above provide a preliminary theoretical framework.

Additional experiments to complete series "B" are currently underway to test the remaining treatments - 2% CO_2 with low Fe, and air with high Fe. These experiments are necessary to verify the results of series "A" experiments at high CO_2 in which Scenedesmus appears to have won.

We will also test the hypothesis that growth rate is an index of competitive ability by diverting all monospecific effluent from the competition vessel after completion of an experimental run. Complete diversion will eliminate cell replacement and create a conventional situation in which one species washes out of the vessel.

REFERENCES

Morel, F.M.M., J.G. Roeter, D.M. Anderson, and R.R.L. Guillard. 1979. Aquil: a chemically defined phytoplankton culture medium for trace metals studies. J. Phycol. 15: 135-141.

Rippka, R., J. Dervelles, J.B. Waterbury, M. Herdman and R.Y. Stanier. 1979. Generic assignments, strain, histories, and properties of pure cultures of Cyanobacteria. J. Gen. Microbiol. 111: 1-61.

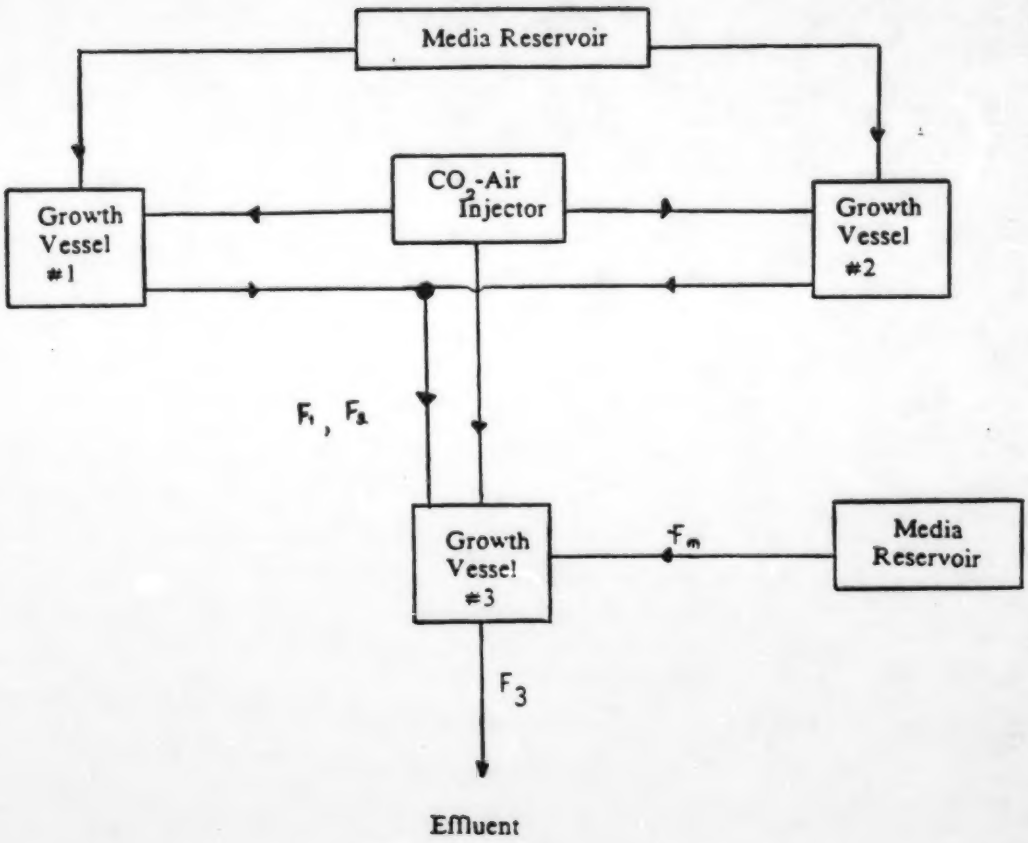

Figure 1. Schematic diagram of continuous culture apparatus. Two algal species are grown in monospecific cultures (vessels #1 and 2) with effluent converging downstream in vessel #3. F_1, F_2 and F_m are the individual media flow rates into vessel #3.

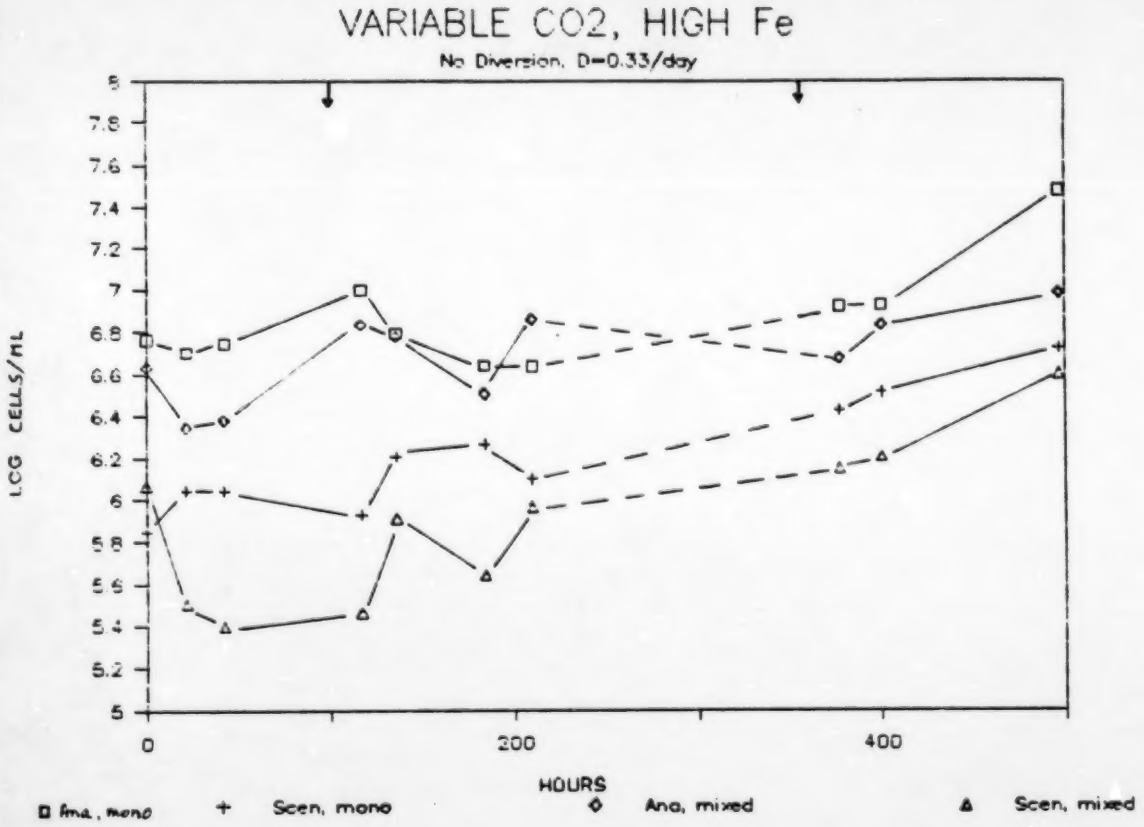

Figure 2. Anabaena and Scenedesmus cell concentrations in series "A" monospecific and mixed cultures at high Fe (pFe 17) in air, 0.1% and 1% CO_2. The arrows indicate shifts in CO_2 (from air to 0.1% at 100 hr, and from 0.1% to 1% at 354 hr).

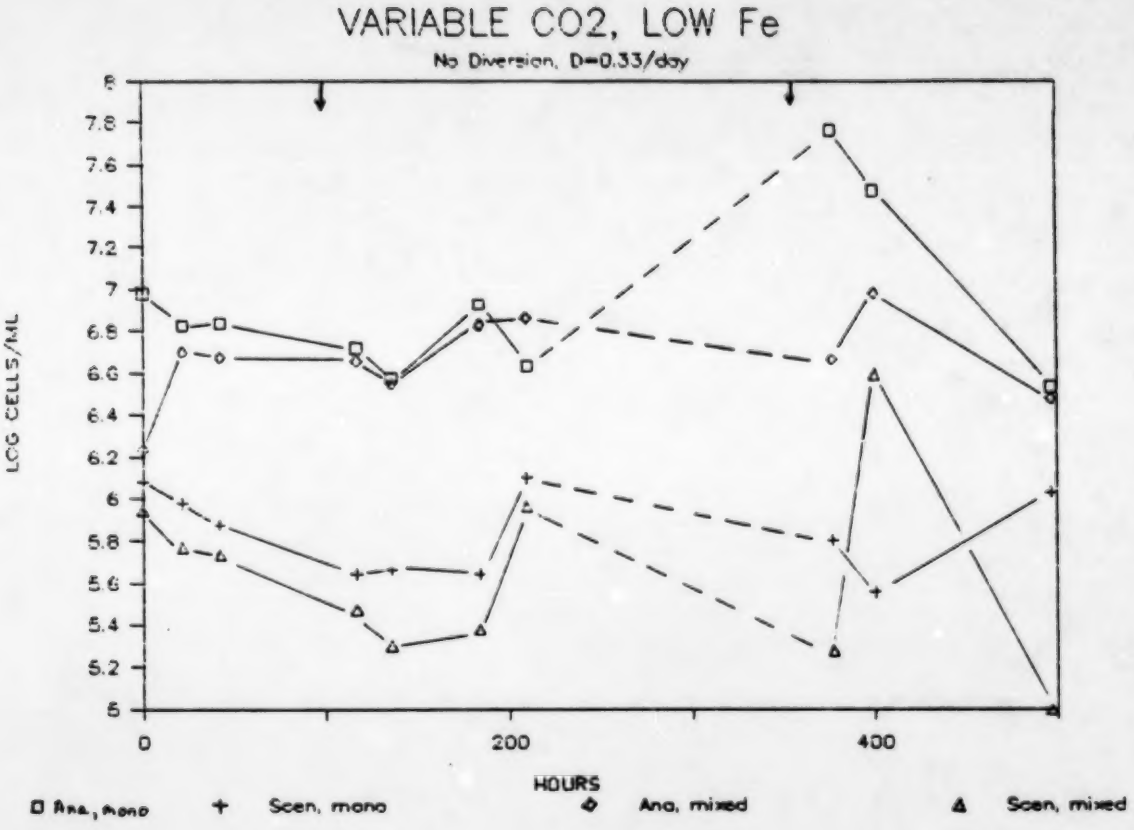

Figure 3. Anabaena and Scenedesmus cell concentrations in series "A" monospecific and mixed cultures at low Fe (pFe 19) in air, 0.1% and 1% CO_2. The arrows indicate shifts in CO_2 (from air to 0.1% at 100 hr, and from 0.1% to 1% at 354 hr).

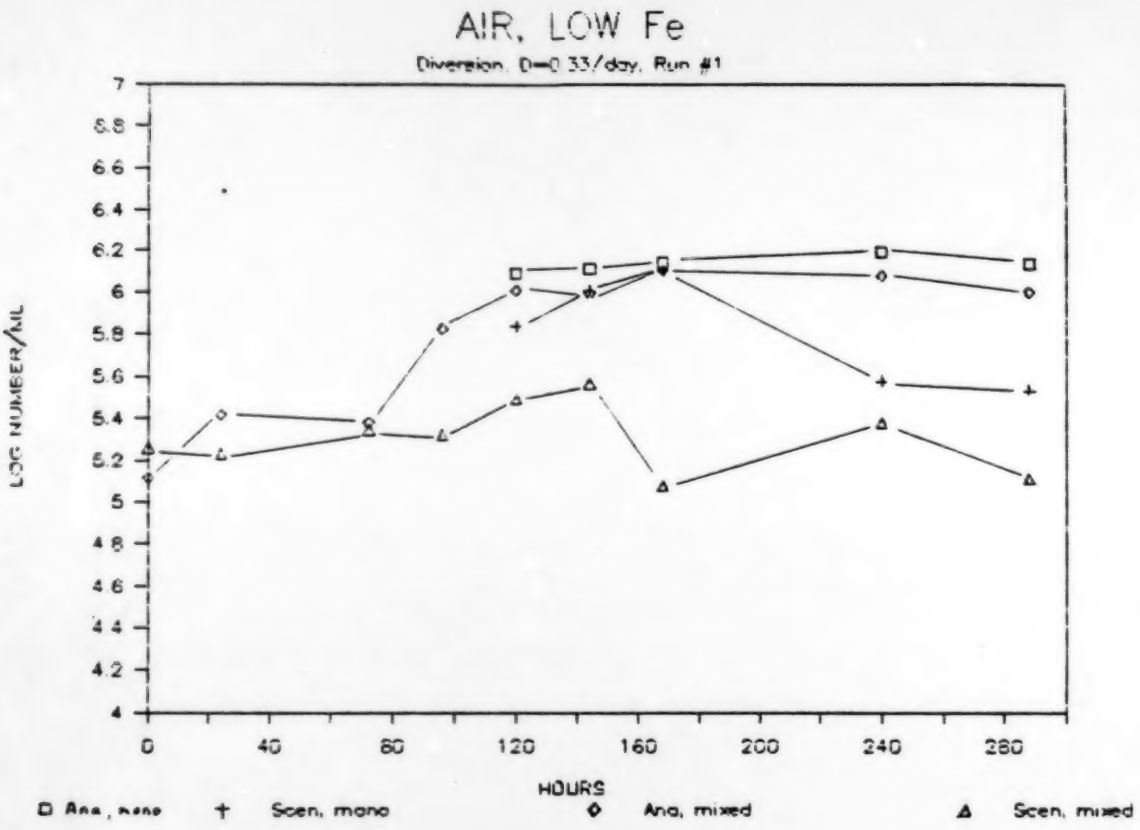

Figure 4. Anabaena and Scenedesmus cell concentrations in series "B" run #1 monospecific and mixed cultures at low Fe (pFe 19) in air.

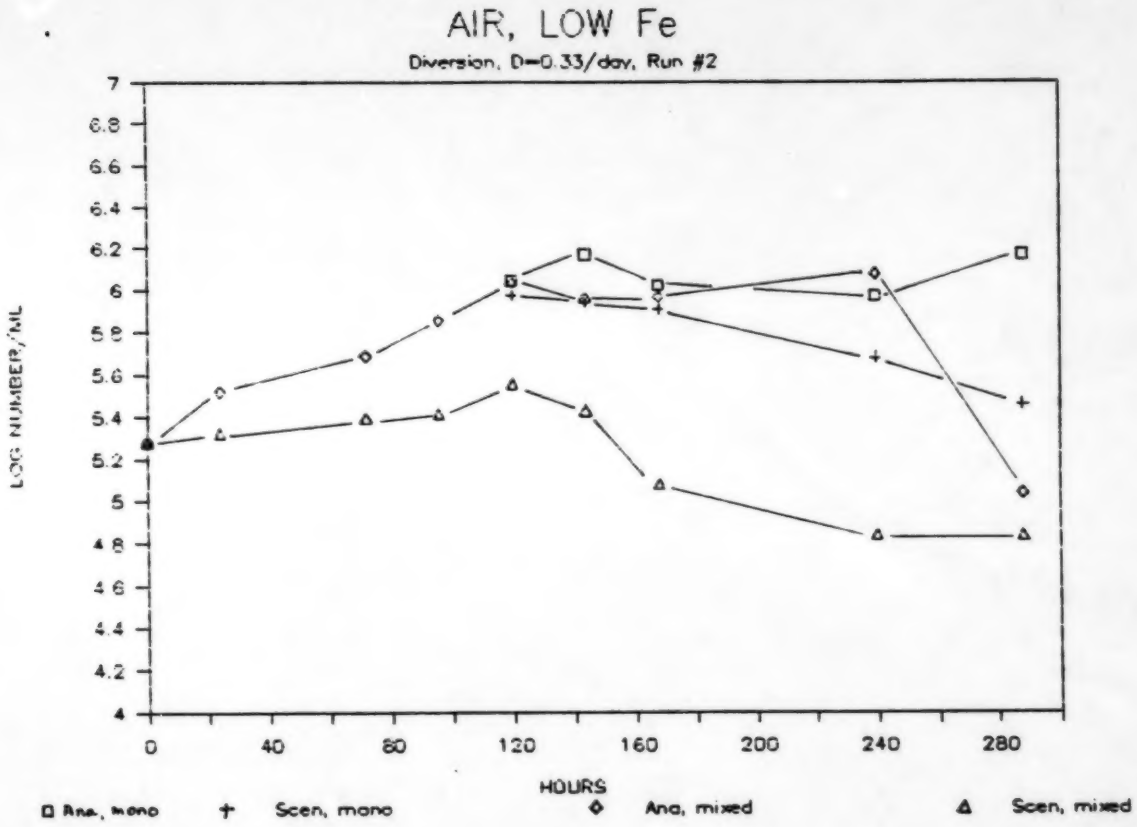

Figure 5. Anabaena and Scenedesmus cell concentrations in series "B" run #2 monospecific and mixed cultures at low Fe (pFe 19) in air.

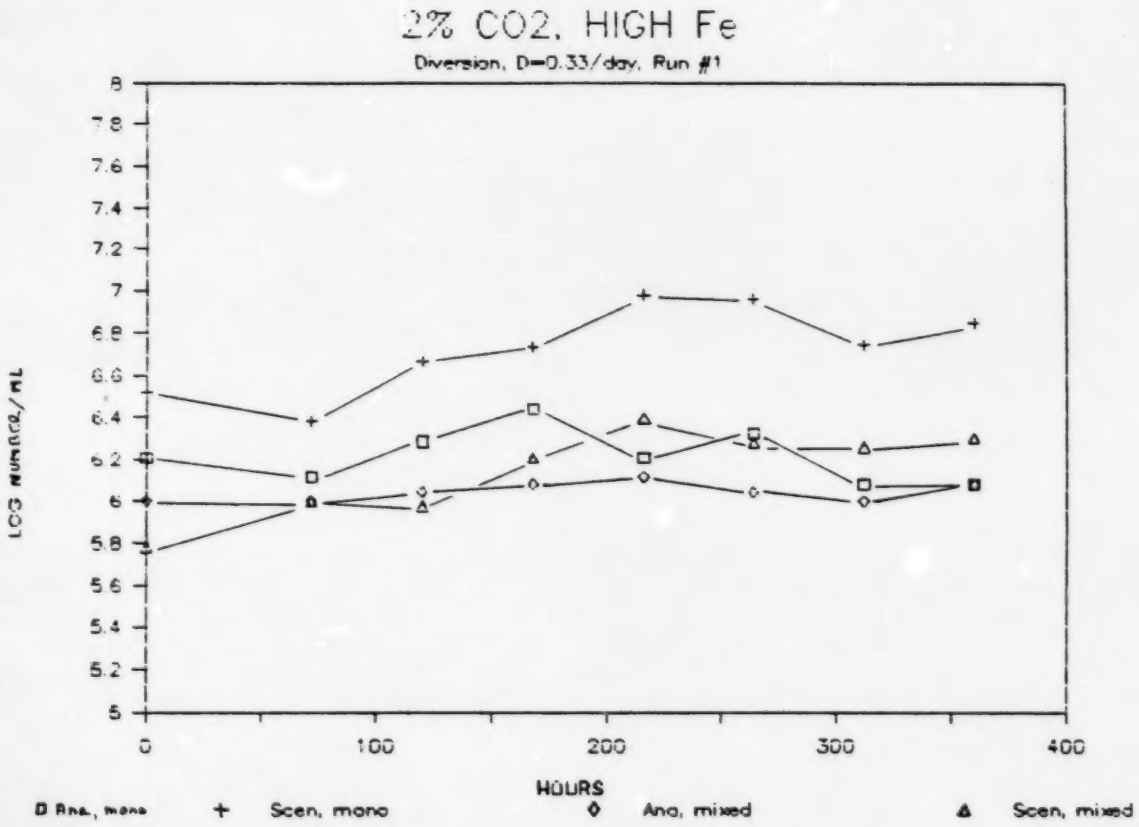

Figure 6. Anabaena and Scenedesmus cell concentrations in series "B" run #1 monospecific and mixed cultures at high Fe (pFe 17) in air enriched to 2% CO_2.

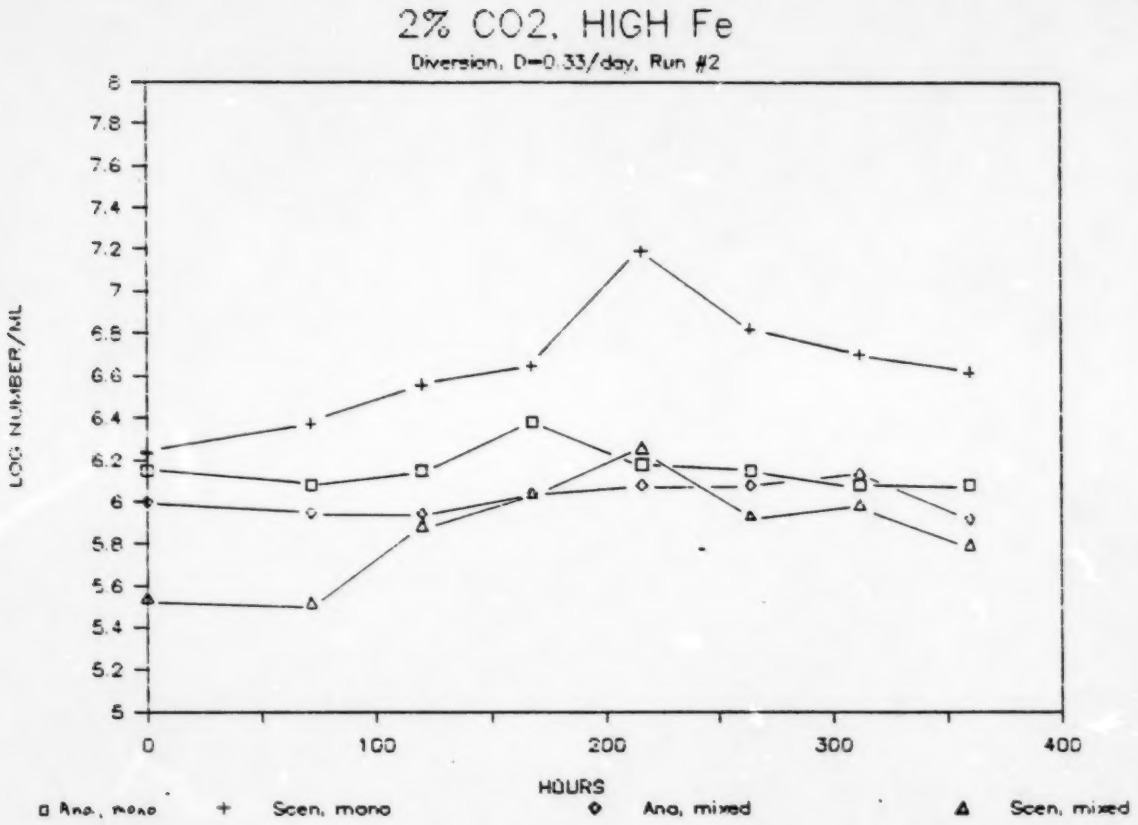

Figure 7. Anabaena and Scenedesmus cell concentrations in series "B" run #2 monospecific and mixed cultures at high Fe (pFe 17) in air enriched to 2% CO_2.

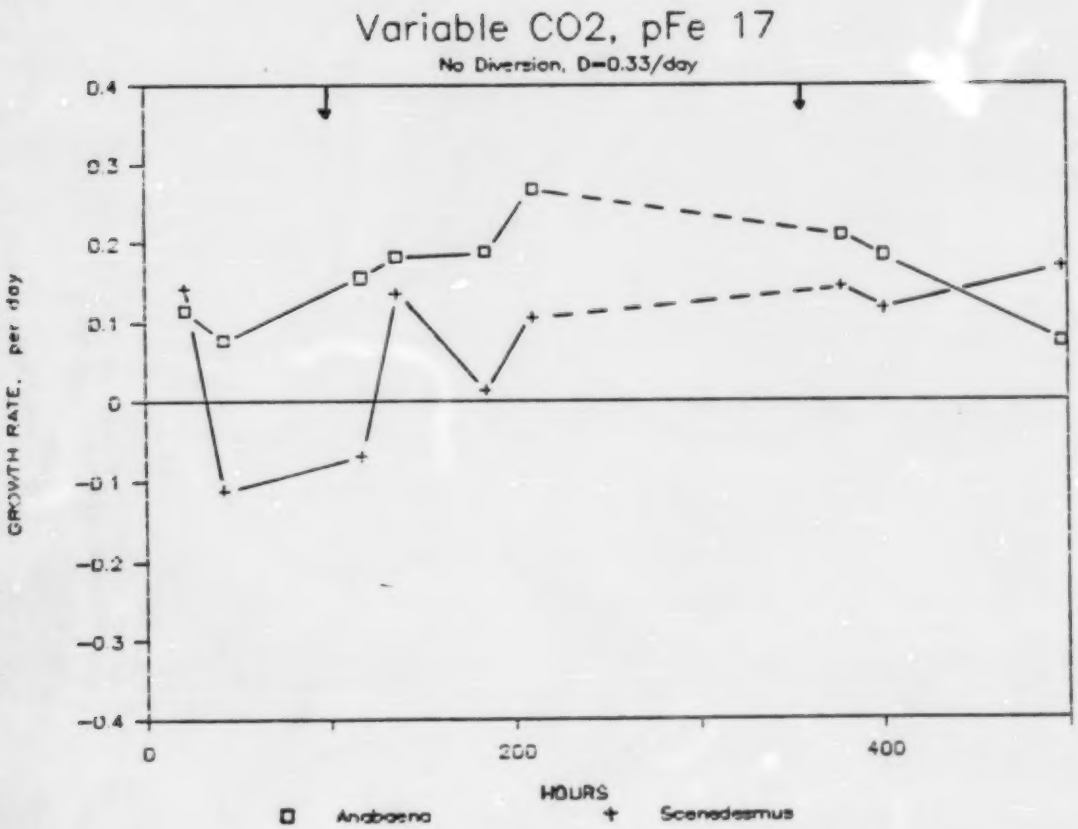

Figure 8. Anabaena and Scenedesmus growth rates in series "A" mixed culture at high Fe (pFe 17) in air, 0.1% and 1% CO_2. The arrows indicate shifts in CO_2 (from air to 0.1% at 100 hr, and from 0.1% to 1% at 354 hr).

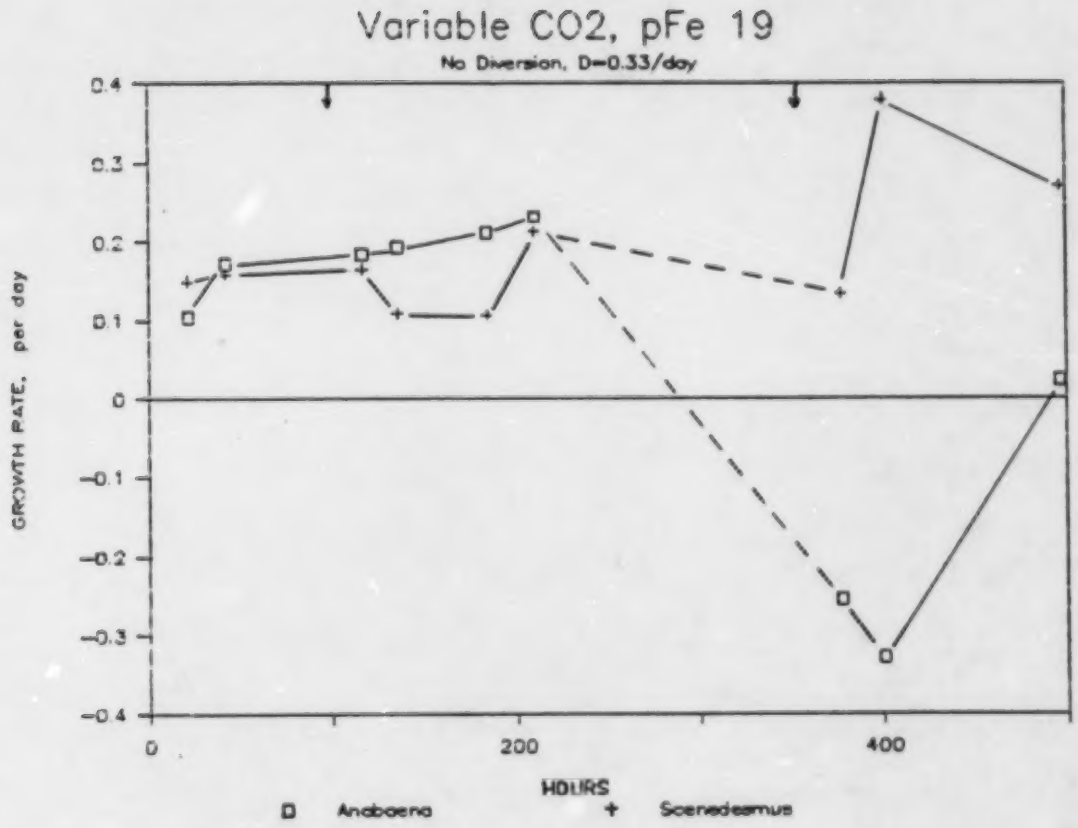

Figure 9. Anabaena and Scenedesmus growth rates in series "A" mixed culture at low Fe (pFe 19) in air, 0.1% and 1% CO_2. The arrows indicate shifts in CO_2 (from air to 0.1% at 100 hr, and from 0.1% to 1% at 354 hr).

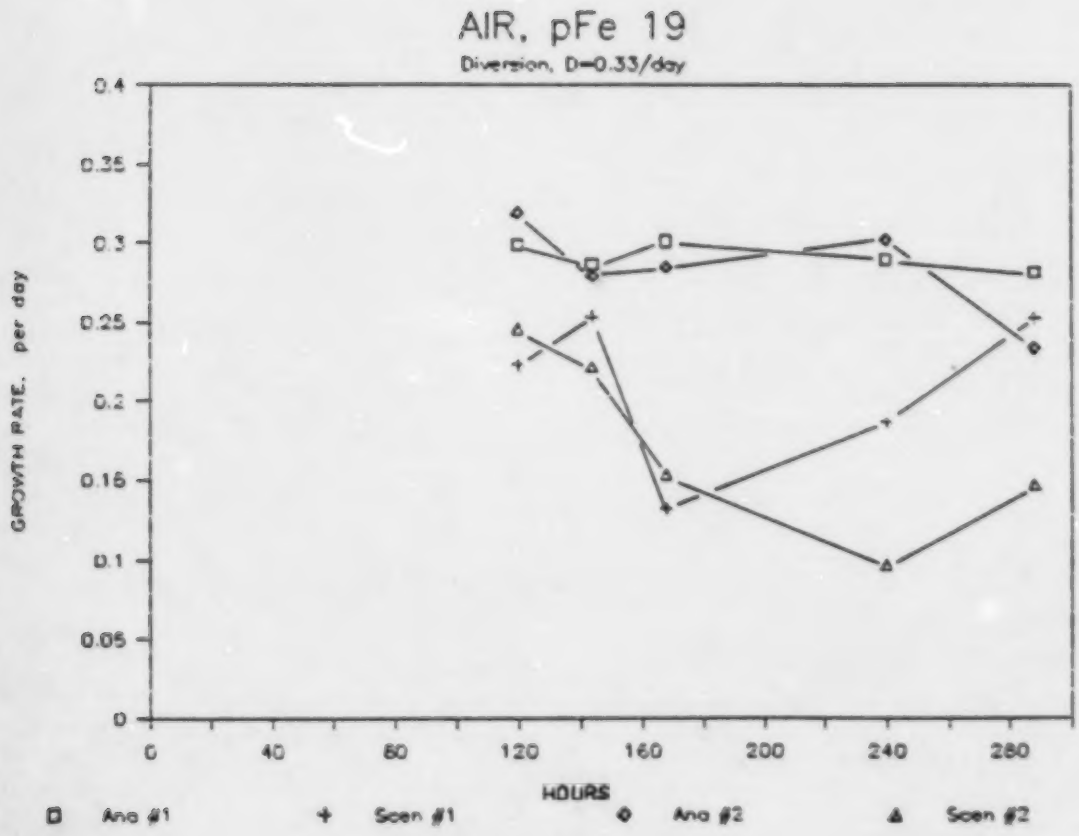

Figure 10. Anabaena and Scenedesmus growth rates in series "B" mixed cultures (runs #1 and 2) at low Fe (pFe 19) in air.

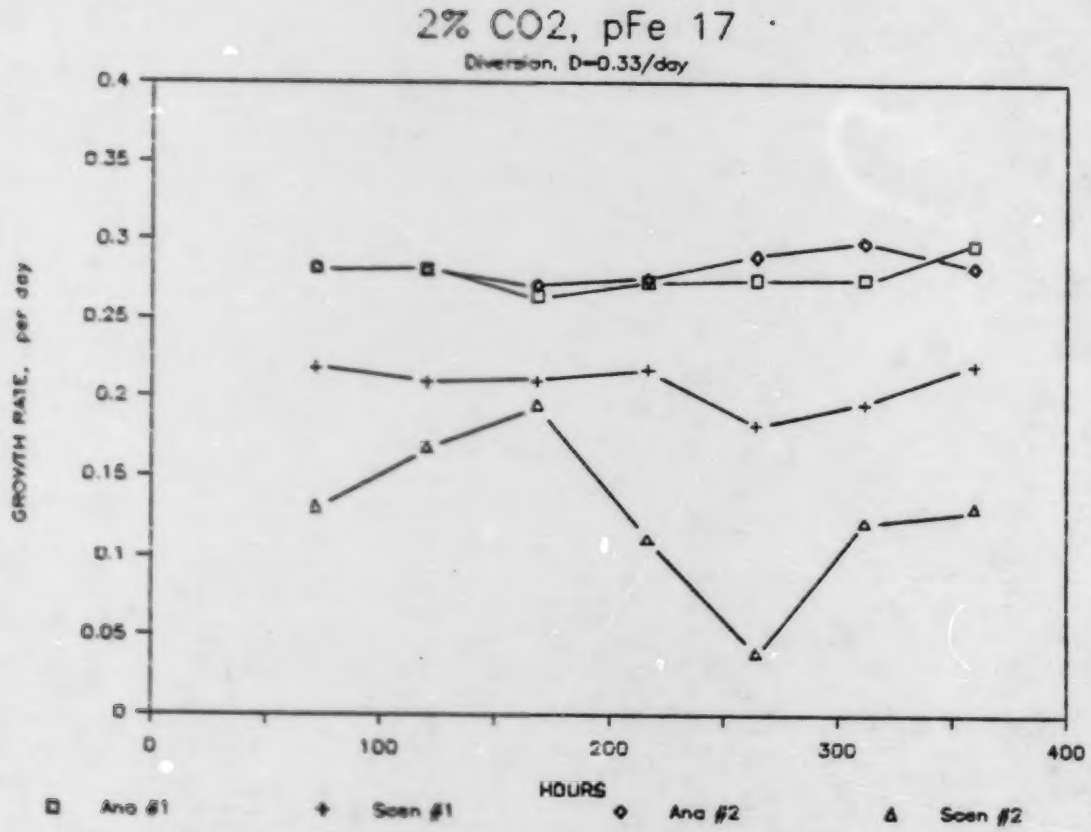

Figure 11. Anabaena and Scenedesmus growth rates in series "B" mixed cultures (runs #1 and 2) at high Fe (pFe 17) in air enriched with 2% CO_2.